

CLAIMS

1. A method of distinguishing between at least 25 different serotypes of *Streptococcus pneumoniae* in a sample, the method comprising,
 - 5 i) analysing at least a portion of the nucleotide sequence between the 3' end of the *cpsA* gene and the 5' end of the *cpsB* gene, and/or
 - ii) analysing at least a portion of the *wzy* and/or *wzx* gene(s).
2. The method of claim 1 which distinguishes between at least 70 different
10 serotypes of *Streptococcus pneumoniae* in a sample.
3. A method of determining the serotype of *Streptococcus pneumoniae* in a sample, the method comprising,
 - 15 i) analysing at least a portion of the nucleotide sequence between the 3' end of the *cpsA* gene and the 5' end of the *cpsB* gene, and/or
 - ii) analysing at least a portion of the *wzy* and/or *wzx* gene(s),wherein the serotype is selected from the group consisting of: 2, 7A, 7B, 7C, 9A, 9L, 10F, 10A, 10B, 10C, 11F, 11A, 11B, 11C, 11D, 12F, 12A, 12B, 13, 15F, 15A, 15B, 15C, 16A, 17F, 17A, 18F, 18A, 18B, 21, 22F, 22A, 24F, 24A, 24B, 25F, 25A, 27, 28F,
20 28A, 31, 32F, 32A, 33F, 33A, 33B, 33C, 33D, 34, 35A, 35B, 35C, 36, 37, 38, 39, 40, 41F, 41A, 42, 43, 44, 45, 46, 47, 47A and 48.
4. A method of determining the serotype of *Streptococcus pneumoniae* in a sample, the method comprising analysing at least a portion of the nucleotide sequence
25 between the 3' end of the *cpsA* gene and the 5' end of the *cpsB* gene.
5. The method of claim 4, wherein the portion of the nucleotide sequence between the 3' end of the *cpsA* gene and the 5' end of the *cpsB* gene which is analysed is any nucleotide which is polymorphic between at least some of the *S. pneumoniae* serotypes
30 referred to in Figure 2.
6. The method of claim 4 or claim 5, wherein the method comprises amplifying at least a portion of the nucleotide sequence between the 3' end of the *cpsA* gene and the 5' end of the *cpsB* gene, and sequencing the amplification product.
35
7. The method of claim 6, wherein the entire approximate 800 bp region as provided in Figure 2 is amplified and sequenced.

8. The method of claim 7, wherein the amplification is performed using primer pairs comprising a sequence selected from the group consisting of:

1) GGCATT(/C)TATGGAGTTGATTCTG(/A)TCCATT(/C)CACAC(C/T)TTAG
(SEQ ID NO:68) and

5 GC(/T)TCAATG(/A)TGG(/A)GCAATG(/T)ACTGGA(/C)GTA(/G)ATTCCCA(/G)A
CATC (SEQ ID NO:73),

2) GGCATT(/C)TATGGAGTTGATTCTG(/A)TCCATT(/C)CACACC(/T)
TTAG (SEQ ID NO:68) and
CCATCAC(/T)ATAGAGGTTAC(/A)TG(/A)TCTGGCATT(/C)GC (SEQ ID NO:71),

10 3) GAAAGTGGG(/A/T)GGG(/A/T)A(/G)A(/C)T(/G)TAT(/C)AAAGTA(/G)
AATTCT(/G)CAAGAT(/C)TTA(/G)AAA(/G)G (SEQ ID NO:70) and
T(/G)CATG(/A)CTA(/G)AAC(/T)TCT(/A)ATC(/T)AAG(/A)GCATAACGACTATC(/
T) (SEQ ID NO:72), and

15 4) primer pairs that amplify the same region, or diagnostic portion thereof, from
the genome of a strain of *S. pneumoniae* as the primers provided in 1) to 3).

9. The method of claim 4, wherein the nucleotide sequence analysis step comprises
determining whether a polynucleotide obtained from *S. pneumoniae* selectively
hybridises to a polynucleotide probe comprising one or more polymorphic regions of
20 the nucleotide sequence between the 3' end of the *cpsA* gene and the 5' end of the *cpsB*
gene, wherein such polymorphic regions are shown in Figure 2.

10. The method of claim 9, wherein the nucleotide sequence analysis step comprises
a plurality of said polynucleotide probes.

25 11. The method of claim 9 or claim 10, wherein the polynucleotide probe(s) is
present as a microarray.

12. A method of determining the serotype of *Streptococcus pneumoniae* in a
30 sample, the method comprising analysing at least a portion of the *wzy* and/or *wzx*
gene(s).

13. The method of claim 12 which comprises amplifying at least a portion of the
wzy and/or *wzx* gene(s), and determining the length of the amplification product.

35 14. The method of claim 13, wherein at least a portion of the *wzy* and/or *wzx* gene(s)
is amplified using a primer comprising a sequence selected from any one of SEQ ID

NO's 75 to 139 or 144 to 333, or a primer that can be used to amplify the same region, or diagnostic portion thereof, from the genome of a strain of *S. pneumoniae* as a primers provided as any one of SEQ ID NO's 75 to 139 or 144 to 333.

- 5 15. A method of determining the serotype of *Streptococcus pneumoniae* in a sample, the method comprising performing a method according to any one of claims 4 to 11, and the method according to any one of claims 12 to 14.
16. A method of identifying serotype 3 of *Streptococcus pneumoniae* in a sample,
10 the method comprising a method according to any one of claims 4 to 11, and analysing the *orf2 (wze)-cap3A-cap3B* region.
17. The method of claim 16, wherein the *orf2 (wze)-cap3A-cap3B* region is analysed by amplifying a portion of the *orf2 (wze)-cap3A-cap3B* region using primer
15 pairs selected from the group consisting of:
 - 1) GCACAAAAAAAAGTTTGATATTTCCCTTGACAATAG (SEQ ID NO:140) and GCAGGATCTAAGGAGGCTTCAAGATTCAACTC (SEQ ID NO:141),
 - 2) CGAACCTACTATTGAGTGTGATACTTTTATGGGATACAGAG (SEQ
20 ID NO:142) and CTGACAGCATGAAAATATATAACCGCCCAACGAATAAG (SEQ ID NO:143), and
 - 3) primer pairs that amplify the same region, or diagnostic portion thereof, from the genome of a strain of *S. pneumoniae* as the primers provided in 1) or 2)
- 25 18. The method according to any one of claims 1 to 17, the method further comprising detecting any serotype of *Streptococcus pneumoniae* in the sample.
19. The method of claim 18, wherein the *psaA* and/or pneumolysin genes, or a portion thereof, is amplified.
30
20. The method of claim 19, wherein a portion of the *psaA* gene is amplified using primers comprising the sequence
TACATTACTCGTTCTCTTTCTTTCTGCAATCATTCTTG (SEQ ID NO:64) and
TAGTAGCTGTGCGCTTCTTTACCTTGTTCTGC (SEQ ID NO:65), or primer pairs
35 that amplify the same region, or diagnostic portion thereof, from the genome of a strain of *S. pneumoniae* as SEQ ID NO:64 and SEQ ID NO:65.

21. The method of claim 19, wherein a portion of the pneumolysin gene is amplified using primers comprising the sequence AGAATAATCCCACTCTTCTTGCGGTTGA (SEQ ID NO:66) and CATGCTGTGAGCCGTTATTTTTTCATACTG (SEQ ID NO:67) or primer pairs that amplify the same region, or diagnostic portion thereof, from the genome of a strain of *S. pneumoniae* as SEQ ID NO:66 and SEQ ID NO:67.
22. An isolated polynucleotide comprising a sequence of nucleotides selected from those provided as SEQ ID NO's 2 to 63, or a fragment thereof which is at least 10 nucleotides in length, with the proviso that the polynucleotide does not comprise the entire *wzy* and/or *wzx* gene(s) of a *S. pneumoniae* serotype selected from the group consisting of: 1, 2, 4, 6A, 6B, 8, 9V, 14, 18C, 19F, 19A, 19B, 23F, 33F and 37, or the entire *wzx* gene of *S. pneumoniae* serotype 19C.
23. An isolated polynucleotide comprising a sequence of nucleotides selected from the group consisting of: 1-AF532632, 10A-AF532633, 10A-AF532634, 10B-AY508586, 10F-AF532635, 10F-AF532636, 10F-AY508587, 11A-AF532637, 11A-AF532638, 11B-AF532639, 11C-AY508588, 11C-AY508589, 12A-AY508590, 12A-AY508591, 12F-AF532640, 12F-AF532641, 13-AF532642, 14-AF532643, 14-AF532644, 14-AF532645, 15A-AF532646, 15A-AF532647, 15B-AF532648, 15B-AF532649, 15B-AF532650, 15C-AF532651, 15C-AF532652, 15C-AY330714, 15C-AY330715, 15C-AY508592, 15C-AY508593, 15F-AY508594, 15F-AY508595, 16A-AY508596, 16F-AF532653, 16F-AF532654, 17A-AF532655, 17A-AY508597, 17F-AF532656, 17F-AF532657, 18A-AF532658, 18A-AF532659, 18B-AF532660, 18C-AF532661, 18F-AF532662, 18F-AY330716, 18F-AY508598, 19A-AF532663, 19A-AF532664, 19B-AY508599, 19C-AY508600, 19C-AY508601, 19F-AF532665, 19F-AF532666, 19F-AF532667, 19F-AF532668, 2-AF532669, 20-AF532670, 21-AF532671, 21-AY508602, 22A-AF532672, 22F-AF532673, 23A-AF532674, 23A-AF532675, 23B-AF532676, 23B-AY330717, 23F-AF532677, 23F-AF532678, 23 F-AF532679, 24A-AY508603, 24B-AY508604, 24F-AY508605, 24F-AY508606, 24F-AY508607, 25F-AF532711, 27-AY508608, 28A-AY508609, 28F-AY508610, 28F-AY508611, 29-AF532680, 29-AY330718, 3-AF532681, 3-AF532682, 3-AF532683, 31-AF532684, 32A-AY508612, 32A-AY508613, 32F-AY508614, 33A-AF532685, 33B-AF532686, 33B-AY508615, 33C-AY508616, 33F-AF532687, 33F-AF532688, 33F-AF532689, 34-AF532690, 35A-AY508617, 35B-AF532691, 35C-AY508618, 35F-AF532692, 36-AY508619, 37-AF532713, 38-AF532712, 39-AY508620, 39-AY508621, 4-AF532693, 40-AY508622, 41A-AY508623, 41F-AY508624, 42-AY508625, 43-AY508626, 45-AY508628, 46-AY508629, 47A-AY508630, 47F-

- AY508631, 48-AY508632, 48-AY508633, 5-AF532696, 5-AF532697, 5-AY508634, 6A-AF532698, 6A-AF532699, 6A-AF532700, 6A-AF532701, 6A-AF532702, 6A-AY508641, 6B-AF532703, 6B-AF532704, 6B-AF532705, 7A-AY508635, 7B-AY508636, 7C-AF532706, 7F-AF532707, 8-AF532708, 9A-AY508637, 9L-AY508638, 9N-AF532709, 9V-AF532710 and 9V-AY508639 as provided in Figure 2, or a fragment thereof which is at least 10 nucleotides in length, with the proviso the polynucleotide does not comprise the 3' end of the *cpsA* gene to the 5' end of the *cpsB* gene of a *S. pneumoniae* serotype selected from the group consisting of: 1, 2, 3, 4, 6A, 6B, 8, 9V, 14, 18C, 19F, 19A, 23F, 33F and 37.
24. An isolated polynucleotide consisting essentially of 10 to 50 contiguous nucleotides corresponding to a portion of the 3' end of the *cpsA* *S. pneumoniae* gene or the 5' end of the *cpsB* *S. pneumoniae* gene.
25. A polynucleotide consisting essentially of 10 to 50 contiguous nucleotides corresponding to a portion of the *S. pneumoniae* *wzy* and/or *wzx* gene(s).
26. The polynucleotide of claim 24 or claim 25, wherein said polynucleotide comprises one or more nucleotides which differ between different *S. pneumoniae* serotypes.
27. The polynucleotide of claim 26, wherein the nucleotides which differ between *S. pneumoniae* serotypes correspond to one or more of positions as shown in Figure 2.
28. A composition comprising a plurality of polynucleotides according to any one of claims 22 to 27 and an acceptable carrier or excipient.
29. A microarray comprising a plurality of polynucleotides according to any one of claims 22 to 27.
30. The use of a microarray according to claim 29 for serotyping a strain of *S. pneumoniae*.
31. A kit comprising at least one polynucleotide according to any one of claims 22 to 27.